ATENT COOPERATION TR TY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

NILSSON, Brita
AB Stockholms Patentbyrå, Zacco & Bruhn
Box 23101
S-104 35 Stockholm
SUÈDE

Date of mailing (day/month/year) 02 February 2001 (02.02.01)	
Applicant's or agent's file reference 192971101/BN	**IMPORTANT NOTIFICATION**
International application No. PCT/EP00/01038	**International filing date** (day/month/year) 09 February 2000 (09.02.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

PHARMATRIX AB
Spelmanshöjden 14
S-174 50 Sundbyberg
Sweden

State of Nationality	State of Residence
SE	SE

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

EUROCINE AB
Karolinska Institute
Science Park
Fogdevreten 2
S-171 77 Stockholm
Sweden

State of Nationality	State of Residence
SE	SE

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

simile No.: (41-22) 740.14.35

Authorized officer

C. Cupello

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 December 2000 (14.12.00)	
International application No. PCT/EP00/01038	**Applicant's or agent's file reference** 192971101/BN
International filing date (day/month/year) 09 February 2000 (09.02.00)	**Priority date** (day/month/year) 12 February 1999 (12.02.99)
Applicant SCHRÖDER, Ulf et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

06 September 2000 (06.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
192971101/BN		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/01038	09/02/2000	12/02/1999
International Patent Classification (IPC) or national classification and IPC A61K39/00		
Applicant [PHARMATRIX AB] A EUROCINE AB		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/09/2000	14.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hoesel, H Telephone No. +49 89 2399 8693

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01038

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-10 as originally filed

Claims, No.:

1-10 as received on 25/01/2001 with letter of 24/01/2001

Drawings, sheets:

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10 for IA.

because:

☒ the said international application, or the said claims Nos. 10 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1 - 10

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1 - 10
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1 - 9
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: WO-A-97/47320
D2: WO-A-97/35613

SECTION III:

1. Claim 10 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of its subject-matter (Article 34(4)(a)(i) PCT).

SECTION V:

2. The particular composition comprising a selected immunogen and a particular adjuvant is not disclosed in the prior art referred to in the international search report. The subject-matter of claims 1 - 10 is therefore novel in the sense of Art. 33(2) PCT.

   The closest prior art of D1 and D2 does furthermore not suggest the combination of the adjuvant with antigenically active carbohydrates derived from *M. tuberculosis*. Having regard to the particular characteristics of mycobacterial cell wall constituents and mycobacterial infections, a skilled person would not obviously transfer the teaching of D2 to the preparation and use of mycobacterial immunogens.

   Thus, the claims are considered to be inventive in the sense of Art. 33(2) PCT.

3. For the assessment of the present claim 10 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such

a compound for the manufacture of a medicament for a new medical treatment.

SECTION VII:

4. The description is not in conformity with the claims as required by Rule 5.1(a)(iii) PCT.

SECTION VIII:

5. Claim 2 is not clear with respect to the unexplained abbreviation "LAM" in the structural formula (Art. 6 PCT)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01038

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/39 A61K39/385

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 47320 A (PHARMATRIX AB) 18 December 1997 (1997-12-18) page 4, line 15 -page 5, line 31 page 7, line 6 -page 7, line 9 abstract ---	1-10
Y	WO 97 35613 A (SVENSON STEFAN) 2 October 1997 (1997-10-02) page 2, line 10 -page 4, line 17 abstract ---	1-7
Y	EP 0 448 126 A (NEW YORK BLOOD CENTER INC) 25 September 1991 (1991-09-25) page 16, line 4 - line 11 page 16, line 28 - line 30 page 19, line 28 - line 30 page 19, line 58 page 20, line 28 - line 29 --- -/--	8-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2000	27. 07. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	C.O. Gustafsson

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	ULF SCHRÖDER ET AL: "Nasal and parenteral immunizations with diphtheria toxoid using monoglyceride/fatty acid lipid suspensions as adjuvants" VACCINE, vol. 17, 1999, pages 2096-2103, XP002138680 see abstract -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/01038

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9747320 A	18-12-1997	AU 3199897 A	07-01-1998
		CA 2258017 A	18-12-1997
		EP 0918541 A	02-06-1999
WO 9735613 A	02-10-1997	AU 2027797 A	17-10-1997
		EP 0894008 A	03-02-1999
EP 0448126 A	25-09-1991	US 4847080 A	11-07-1989
		AT 123035 T	15-06-1995
		AT 175121 T	15-01-1999
		AU 622115 B	02-04-1992
		AU 2010088 A	17-11-1988
		AU 2010288 A	17-11-1988
		AU 591054 B	30-11-1989
		AU 3959785 A	12-09-1985
		CA 1250100 A	14-02-1989
		DE 3588022 D	29-06-1995
		DE 3588022 T	05-10-1995
		DE 3588203 D	11-02-1999
		DE 3588203 T	17-06-1999
		DK 103085 A	08-09-1985
		EP 0154902 A	18-09-1985
		IE 75712 B	24-09-1997
		JP 2960064 B	06-10-1999
		JP 61147165 A	04-07-1986
		KR 9008007 B	29-10-1990
		US 5565548 A	15-10-1996
		US 5620844 A	15-04-1997
		US 5158769 A	27-10-1992
		US 5204096 A	20-04-1993
		ZA 8501266 A	30-10-1985
		US 4861588 A	29-08-1989

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
192971101/BN		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/01038	09/02/2000	12/02/1999
Applicant		
PHARMATRIX AB		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

VACCINE FORMULATION COMPRISING MONOGLYCERIDES OR FATTY ACIDS AS ADJUVANT

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/01038

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 10

Claim 10 relates to a method of treatment of the human or animal body by surgery or by therapy/ a diagnostic method practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compound(s) / compostion(s).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 August 2000 (17.08.2000)

PCT

(10) International Publication Number
WO 00/47224 A3

(51) International Patent Classification[7]: A61K 39/39, 39/385

(21) International Application Number: PCT/EP00/01038

(22) International Filing Date: 9 February 2000 (09.02.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9900496-2 12 February 1999 (12.02.1999) SE

(71) Applicant *(for all designated States except US)*: **PHARMATRIX AB** [SE/SE]; Spelmanshöjden 14, S-174 50 Sundbyberg (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SCHRÖDER, Ulf** [SE/SE]; Spelmanshöjden 14, S-174 50 Sundbyberg (SE). **SVENSON, Stefan** [SE/SE]; Brättnevägen 12, S-122 43 Enskede (SE).

(74) Agents: NILSSON, Brita et al.; AB Stockholms Patentbyrå, Zacco & Bruhn, Box 23101, S-104 35 Stockholm (SE).

(81) Designated States *(national)*: AU, CA, JP, NZ, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
14 December 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/47224 A3

(54) Title: VACCINE FORMULATION COMPRISING MONOGLYCERIDES OR FATTY ACIDS AS ADJUVANT

(57) Abstract: A vaccine formulation against a microorganism is disclosed. The formulation comprises: as adjuvant, one or more substances selected from a) monoglyceride preparations having at least 80 % monoglyceride content and b) fatty acids of the general formula CH_3-$(CH^2)_n$-COOH where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from said microorganism which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC). The vaccine formulation is e.g. against *Mycobacterium tuberculosis* and in that case the formulation may comprise, as adjuvant, a mixture of mono-olein and oleic acid, and possibly soybean oil, and, as immunizing component, lipoarabinomamman-tetanus toxoid (LAM-TT).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01038

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/39 A61K39/385

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 47320 A (PHARMATRIX AB) 18 December 1997 (1997-12-18) page 4, line 15 -page 5, line 31 page 7, line 6 -page 7, line 9 abstract ---	1-10
Y	WO 97 35613 A (SVENSON STEFAN) 2 October 1997 (1997-10-02) page 2, line 10 -page 4, line 17 abstract ---	1-7
Y	EP 0 448 126 A (NEW YORK BLOOD CENTER INC) 25 September 1991 (1991-09-25) page 16, line 4 - line 11 page 16, line 28 - line 30 page 19, line 28 - line 30 page 19, line 58 page 20, line 28 - line 29 --- -/--	8-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2000	27. 07. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	C.O. Gustafsson

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01038

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	ULF SCHRÖDER ET AL: "Nasal and parenteral immunizations with diphtheria toxoid using monoglyceride/fatty acid lipid suspensions as adjuvants" VACCINE, vol. 17, 1999, pages 2096-2103, XP002138680 see abstract -----	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/01038

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 10

Claim 10 relates to a method of treatment of the human or animal body by surgery or by therapy/ a diagnostic method practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compound(s) / compostion(s).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/01038

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9747320	A	18-12-1997	AU	3199897 A	07-01-1998
			CA	2258017 A	18-12-1997
			EP	0918541 A	02-06-1999
WO 9735613	A	02-10-1997	AU	2027797 A	17-10-1997
			EP	0894008 A	03-02-1999
EP 0448126	A	25-09-1991	US	4847080 A	11-07-1989
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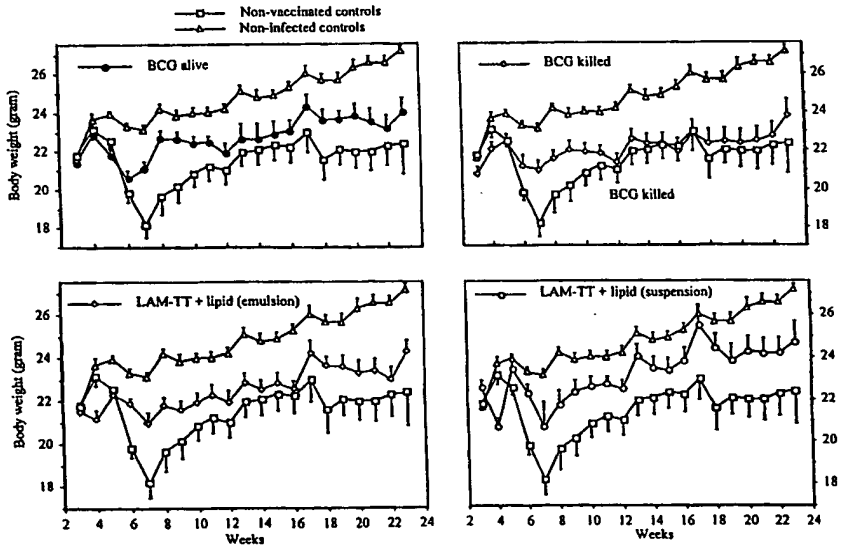

(57) Abstract

A vaccine formulation against a microorganism is disclosed. The formulation comprises: as adjuvant, one or more substances selected from a) monoglyceride preparations having at least 80 % monoglyceride content and b) fatty acids of the general formula $CH_3-(CH^2)_n-COOH$ where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from said microorganism which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC). The vaccine formulation is e.g. against *Mycobacterium tuberculosis* and in that case the formulation may comprise, as adjuvant, a mixture of mono-olein and oleic acid, and possibly soybean oil, and, as immunizing component, lipoarabinomamman-tetanus toxoid (LAM-TT).

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VACCINE FORMULATION

The present invention relates to a novel vaccine formulation against a microorganism e.g. *Mycobacterium tuberculosis*. The preferred route of administration is via the mucosal membranes.

BACKGROUND

The earliest described immunization attempts were carried out in China over 900 years ago, where intranasal inoculation of dried and ground smallpox pustules was performed. In the classical immunology and in combination with vaccination against different types of infectious agents e.g. bacteria, virus or parasites the prevailing dogma has been to administer the vaccine subcutaneously or intramuscularly. However, research has during the last years shown that the body has a very effective immunological system that resides in the mucosa. It has also been shown that you can administer vaccines nasally, orally, rectally and vaginally. In the same way as for the classical immunization it has been shown that by mucosal vaccination there is also a need for enhancement of the immunological response by the addition of adjuvants.

The intranasal route has attracted increased attention because of the greater efficacy in inducing mucosal immune responses than the more conventional regimes of parenteral immunization. Furthermore, the realization that approximately 80% of the immune system reside in the mucosa combined with the fact that an equal percentage of the known pathogens enter our bodies via the mucosal membranes has pushed the interest towards the application of mucosal immunization.

It has also been shown that parenteral vaccines do not induce immune response at mucosal sites. Thus, it is also clear that appropriate stimulation of a mucosal site such as the nose or the gut, can generate immune response at other mucosal sites. As an example, it is possible to apply a vaccine in the nose and obtain an immune response in the vagina. Furthermore, the mucosal immune response is very rapid with onset only hours after being subjected to stimulation by a pathogen, as compared to parenteral immunity having a response time of several days.

Tuberculosis (TB) is one of the major causes of morbidity in the world with an estimated death toll of approximately 3 millions per year. It is estimated that 1/3 of the world's population is infected with TB. To a large extent TB is essentially an

uncontrolled problem despite the use of the Bacille Calmette-Guérin (BCG) vaccine for more than 75 years.

The BCG vaccine consists of a weakened strain of a tuberculosis bacteria taken from a cow in 1908. The original bacteria used today were cultured for 13 years for the purpose of weaken their pathogenic characteristics in order to be used as live bacteria for parenteral vaccination of humans. Basically the same strain is used today as the only vaccine available against TB. Several pharmaceutical companies around the world produce the BCG vaccine. The BCG formulation used today consists of freeze-dried attenuated viable BCG vaccine in one container and another container with physiologically acceptable suspension media. Before administration, the freeze-dried BCG is suspended and subsequently administered by injection to the patient. This procedure which has to be carried out immediately in connection with the vaccination, requires skilled personnel and decent facilities in order to avoid contamination. Unfortunately these criteria are hard to keep up with in the developing countries. Thus, it is estimated that failure to keep to this standard costs about USD 500 millions each year world-wide. Consequently, huge savings could be made both in money and product safety, if a system was available where no mixing of vaccines was needed and where injections could be eliminated, thus eliminating the need for highly skilled personnel and sterile conditions.

In clinical trails around the world, the protective efficacy of the BCG vaccine has been shown to vary between -50% to +80%. This means that certain clinical studies have shown that in fact you enhance instead of diminish your risk of getting the disease after vaccination.

The BCG vaccine works well for children but has more or less no effect on adults. Consequently there are great efforts made in order to achieve a vaccine against TB for the grown-up population. Up to date however, there are no reports in the literature of a TB vaccine that is better than BCG.

Tuberculosis is spread by close person-to-person contact through infectious aerosols. On rare occasions the disease can be acquired by ingestion or skin trauma. This means that the first organ to get into contact with the bacteria during a normal infection is the mucosal surfaces in the lungs.

Adjuvants are a heterogeneous group of substances that enhance the immunological response against an antigen that is administered simultaneously.

Almost all adjuvants used today for enhancement of the immune response against antigens are particles or are forming particles together with the antigen. In the book "Vaccine Design - the subunit and adjuvant approach" (Ed: Powell & Newman, Plenum Press, 1995) almost all known adjuvants are described both regarding their immunological activity and regarding their chemical characteristics. As described in the book more than 80% of the adjuvants tested today are particles or polymers that together with the antigens (in most cases proteins) are forming particles. The type of adjuvants that are not forming particles are a group of substances that are acting as immunological signal substances and that under normal conditions consist of the substances that are formed by the immune system as a consequence of the immunological activation after administration of particulate adjuvant systems.

Using particulate systems as adjuvants, the antigens are associated or mixed with or to a matrix, which has the characteristics of being slowly biodegradable. Of great importance using such matrix systems are that the matrices do not form toxic metabolites. Choosing from this point of view, the main kinds of matrices that can be used are mainly substances originating from a body. With this background there are only a few systems available that fulfill these demands: lactic acid polymers, poly-amino acids (proteins), carbohydrates, lipids and biocompatible polymers with low toxicity. Combinations of these groups of substances originating from a body or combinations of substances originating from a body and biocompatible polymers can also be used. Lipids are the preferred substances since they display structures that make them biodegradable as well as the fact that they are the most important part in all biological membranes.

Lipids are characterized as polar or non-polar. The lipids that are of most importance in the present invention are the polar lipids since they have the capacity to interact and form particulate systems in water. Another way of defining these lipids are as amphiphilic due to their chemical structure with one hydrophobic and one hydrophilic part in the molecule thereby being useable as surface active substances. Examples of main groups of polar lipids are mono-glycerides, fatty acids, phospholipids and glycosphingolipids. These main groups can be further characterized depending on the length of the acyl chain and the degree of saturation of the acyl chain. Since the number of carbon atoms in the acyl chain can be in the range of 6 to 24, and the number of unsaturated bonds can be varied, there is an almost infinite number of combinations regarding the chemical composition of the lipid.

Particulate lipid systems can be further divided into the different groups as discussed in the scientific literature such as liposomes, emulsions, cubosomes, cochleates, micelles and the like.

In a number of systems the lipids may spontaneously form, or can be forced to form, stabile systems. However, under certain circumstances other surface-active substances have to be introduced in order to achieve stability. Such surface-active systems can be of non-lipid character but possess the characteristics of the polar lipids having hydrophobic and hydrophilic parts in their molecular structure.

Another factor that has been shown to be of importance is that lipids exhibit different physical chemical phases, these phases have in different test systems been shown to enhance uptake of biological substances after administration to mucous membranes. Examples of such physical chemical phases described are L2, lamellar, hexagonal, cubic and L3.

In the same way as within the classical immunology where vaccines (antigens) are administered parenterally, there is within mucosal immunization a great interest in directing the immunological response towards development of humoral and/or cellular response. If you obtain a humoral response it would be important to direct the response in a way that a certain class of antibodies would be obtained. In order to obtain such a goal, specific immune stimulating agents can be added to the formulation of antigens and adjuvants.

A formulation which fulfils these goals is described in PCT/SE97/01003, the contents of which is incorporated herein by reference. The disclosed formulation comprises monoglycerides and fatty acids. The monoglycerides comprise one or more substances selected from monoglycerides wherein the acyl group contains from 6 to 24 carbon atoms, preferably 8 to 20 carbon atoms, even more preferably 14 - 20 carbon atoms and where the acyl chain may contain unsaturated bonds.

The acyl chain of the fatty acid may be varied between 4 and 22, preferably 8 to 18 and where the acyl chain may contain one or more unsaturated bonds. A combination of the monoglyceride mono-olein and oleic acid has shown to be an L3 phase, which can be described as sponge-like structure, in contrast to liposomes that form onion-like lamellar structures.

Said combination of monoglycerides and fatty acids my be further formulated by the addition of a biocompatible and biodegradable oil thus forming an oil in water

(o/w) or w/o/w emulsion. Such emulsions have been shown in the literature to be very effective in enhancing the cellular response against an antigen after administration to an animal (Singh, M., et al 1997, *Vaccine* 15, 1773-78). It is generally accepted that in order to have an acceptable vaccine against TB there is a need for a cellular immune response.

Thus, there is a need for a simple way of administering a vaccine combined with an antigen that is easily documented and formulated. One way of producing such a system would be to use antigenic surface components from bacteria which would have the capacity to provoke an immune response in a body, preferably producing a protective immunity against the pathogen which was the origin of the antigen. A number of such systems are available today, however the majority of these are based on membrane components which are proteins.

Most virulent bacteria have carbohydrates on their surface, such as lipopolysaccharides and capsular polysaccharides. Antibodies directed against capsular polysaccharides provide, among other things, enhanced phagocytosis and killing of bacterial cells. Usually there are a number of serotypes of a given bacterial species, for example there are more than 80 known serotypes of Streptococcus pneumoniae related to their carbohydrate capsular structures.

Bacterial polysaccharides are classical examples of antigens that are not T helper cell-dependent, and hence, if they are immunogenic at all, they mainly induce IgM class of antibodies. This is so, because only B cells respond to them, and B cells cannot mediate the memory function as opposed to the T cells, which also mediate immunological booster effects.

In immunologically immature small children, elderly and immunosuppressed persons polysaccharides are known to be poor immunogens or not at all immunogenic.

Therefore, polysaccharide antigens which are chemically conjugated to carriers comprising T cell epitopes are effective as vaccines also for the above mentioned immunologically immature children and immunosuppressed adults.

The vaccine-producing industry has long been searching for a general method of producing conjugate-type vaccines. A general and simple method to produce such vaccines would not only be more practical but would also make process and quality control easier.

A method which provides a general method of producing immunogenic products comprising antigenically active carbohydrate moieties and immunologically active carriers, producing useful immunizing components in conjugate-type vaccines is described in WO 97/ 35613, the contents of which is incorporated herein by reference.

In said International application is described a method of producing an immunogenic product consisting of antigenically active carbohydrate moieties which are each covalently coupled via identical specified divalent bridge groups to immunologically active carriers containing amino groups.

In a preferred embodiment of said invention, the antigenically active carbohydrate moieties of the immunogenic products derive from bacterial O-polysaccharides and/or capsular polysaccharides. Specific examples of such saccharides are those which derive from Salmonella serotypes BO and/or DO or from different serotypes of Streptococcus pneumoniae capsular polysaccharides or from Haemophilus influenzae capsular polysaccharides.

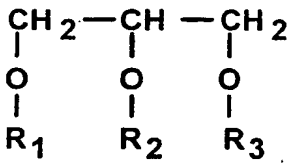
Another carbohydrate moiety, which is of great importance, is the surface carbohydrate from *M. tuberculosis*. This carbohydrate consists of Lipoarabinomannan (LAM) and is the antigenically dominating surface antigen of mycobacteria, and accounts for up to 15 mg/g of the bacterial weight. LAM has profound biologic effects; hence LAM has been reported to interfere with gamma-interferon mediated activation of macrophages, scavenge toxic oxygen free radicals, inhibit protein kinase activity, and induces the expression of macrophage-early genes. LAM has been tested extensively as a possible antigen in vaccine formulations for a long time, however only by parenteral administration, resulting in poor protection in animal experiments.

The immunologically active carriers of the immunogenic product of the conjugate of said invention is preferably derived from polypeptides. In a preferred embodiment said polypeptide is tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

Description of the invention

The present invention is directed to a vaccine formulation against a microorganism comprising, as adjuvant, one or more substances selected from

a) monoglyceride preparations having at least 80 % monoglyceride content and having the general formula



wherein R_1 and R_2 is H and R_3 is one acyl group containing from 6 to 24 carbon atoms, and where the acyl chains may contain one or more unsaturated bonds and

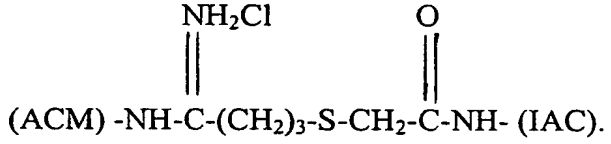
b) fatty acids of the general formula

$$CH_3 - (CH_2)_n - COOH$$

where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and

as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from said microorganism which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC).

In an embodiment of the invention the immunologically active carriers (IAC) contain amino groups and said divalent bridge group has the following structural formula



(ACM) -NH-C-$(CH_2)_3$-S-CH_2-C-NH- (IAC).

The adjuvant of the vaccine formulation of the invention preferably has a monoglyceride preparation content of at least 90 %, preferably at least 95 %, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more unsaturated bonds, and the immunologically active carriers (IAC) are derived from polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

The vaccine formulation according to the invention may further comprise pharmaceutical excipients selected from the group consisting of biocompatible oils, such as such as rape seed oil, sunflower oil, peanut oil, cotton seed oil, jojoba oil,

squalan or squalene, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

A most preferred embodiment of the invention is a vaccine formulation which comprises, as adjuvant, a mixture of mono-olein and oleic acid, and possibly soybean oil, and, as immunizing component, lipoarabinomannan-tetanus toxoid (LAM-TT).

Examples of other antigenically active carbohydrate moieties of the immunogenic products of the immunizing component of the invention derive from bacterial O-polysaccharides and/or capsular polysaccharides. Specific examples of such saccharides are those which derive from Salmonella serotypes BO and/or DO or from different serotypes of Streptococcus pneumoniae capsular polysaccharides or from Haemophilus influenzae capsular polysaccharides.

In another preferred embodiment of the vaccine formulation according to the invention, the formulation is formulated into a preparation for mucosal administration, such as nasal, pulmonary, oral, rectal or vaginal administration.

Another aspect of the invention is directed to an aerosol or spray package comprising a TB vaccine composition according to the invention.

Yet another aspect of the invention is directed to a nose-drop package comprising a TB vaccine composition according to the invention.

A further aspect of the invention is directed to a method of vaccinating a mammal against a microorganism having antigenically active carbohydrate moieties (ACM), which comprises mucosal administration to the mammal of an protection-inducing amount of a TB vaccine composition according to the invention.

As described above the present commercially available vaccine against TB comprises an attenuated strain of the bacteria. Such systems may under certain circumstances, when administered as a vaccine, result in an infection by the attenuated bacteria. Furthermore, antigen systems based on whole bacteria are difficult to standardize according to pharmaceutical regulations. Thus, the preferred system as described in the present invention is a purified antigen from the pathogen, which, in combination with adequate adjuvants results in protective immunity. However, certain antigens, such as carbohydrates, may not generate protective immunity if not associated to a carrier in the form of a conjugate. Furthermore, conjugate vaccines are more stable

and consequently more attractive as antigens/vaccines, especially in the developing world.

The present invention describes a formulation that may be prefabricated, and therefore no need for skilled personnel is needed upon nasal administration, thereby eliminating injection systems such as needles and syringes which in developing world often are contaminated and thus is spreading diseases between patients. Furthermore, a device for multidose aerosol delivery of a nasal vaccine can easily be constructed in way that no person-to-person infection can occur.

The invention will now be illustrated by way of an example, which, however, is not to be interpreted as limitation to the scope of protection according to the appended claims.

Short description of the drawing

Fig. 1 shows the results of the testing disclosed in Example 1.

EXAMPLE 1

Protection of C57BL mice from intranasal sub-lethal challenge with *M. tuberculosis* (MT) by immunization with live BCG and heat-killed BCG or lipoarabinomannan-tetanus toxoid (LAM-TT) conjugate in two different L3 lipid adjuvant formulations.

The emulsion was produced by mixing the LAM-TT conjugate with 100 µl of soybean oil and 100 µl of a mixture of mono-olein and oleic acid (1:1). The amount of LAM-TT conjugate was adjusted so that a dose of 10 µg was given to the mice in 100 µl (parenteral) or in 10 µl (nasal). This mixture was sonicated briefly for a few seconds whereupon 1.0 ml of 0.1 M TRIS buffer and 20 µl of 4 M NaOH were added. Sonication was performed for 2 minutes whereupon the emulsion was used for immunization.

An L3 suspension was produced from a 1:1 molar mixture of mono-olein and oleic acid (1.43 g mono-olein and 1.12 g oleic acid) which was added to 40 ml of 0.1 M Tris buffer. Before sonication for 2 minutes 640 µl of 4 M NaOH was added. Before immunization the L3 adjuvant was mixed with the LAM-TT conjugate in order to achieve a dose of 10 µg in 100 µl for parenteral injection or 10 µl for nasal administration.

Immunization 1; 0 weeks (parenteral for all groups). Immunization 2; 3 weeks (nasally for all groups except live BCG which was administered parenterally). Challange; 4 weeks

Changes of body weight (%) related to initial weight at time 0 weeks. Average body weight changes ± SE of 10 mice/group are shown in Fig.1.

As can be seen from the weight changes, both of the adjuvant formulations containing LAM-TT result in a positive body weight development.

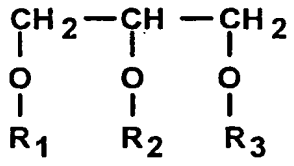
5

Claims

1. Vaccine formulation against a microorganism comprising,

as adjuvant, one or more substances selected from

a) monoglyceride preparations having at least 80 % monoglyceride content and having the general formula



wherein R_1 and R_2 is H and R_3 is one acyl group containing from 6 to 24 carbon atoms, and where the acyl chains may contain one or more unsaturated bonds and

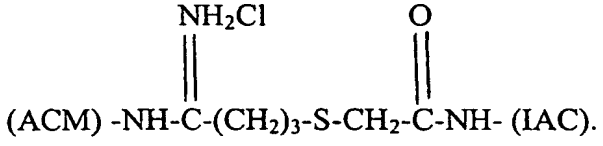
b) fatty acids of the general formula

$$CH_3 - (CH_2)_n - COOH$$

where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and

as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from said microorganism which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC).

2. Vaccine formulation according to claim 1, wherein the immunologically active carriers (IAC) contain amino groups and said divalent bridge group has the following structural formula



(ACM) -NH-C-$(CH_2)_3$-S-CH_2-C-NH- (IAC).

3. Vaccine formulation according to claim 1 or 2, wherein the adjuvant has a monoglyceride preparation content of at least 90 %, preferably at least 95 %, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more

unsaturated bonds, and the immunologically active carriers (IAC) are derived from polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

4. Vaccine formulation according to any one of claims 1 - 3, which further comprises pharmaceutical excipients selected from the group consisting of biocompatible oils, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

5. Vaccine formulation according to claim 3 or 4, wherein the adjuvant is a mixture of mono-olein and oleic acid, and possibly soybean oil, and the immunizing component is lipoarabinomannan-tetanus toxoid (LAM-TT).

6. Vaccine formulation according to any one of claims 1-5, wherein the formulation is formulated into a preparation for mucosal administration.

7. Vaccine formulation according to claim 6, wherein the mucosal administration is selected from nasal, pulmonary, oral, rectal and vaginal administration.

8. Aerosol or spray package comprising a TB vaccine composition according to any one of the claims 1 - 7.

9. Nose-drop package comprising a TB vaccine composition according to any one of the claims 1 - 6.

10. A method of vaccinating a mammal against a microorganism having antigenically active carbohydrate moieties (ACM), which comprises mucosal administration to the mammal of an protection-inducing amount of a TB vaccine composition according to any one of claims 1 - 7.

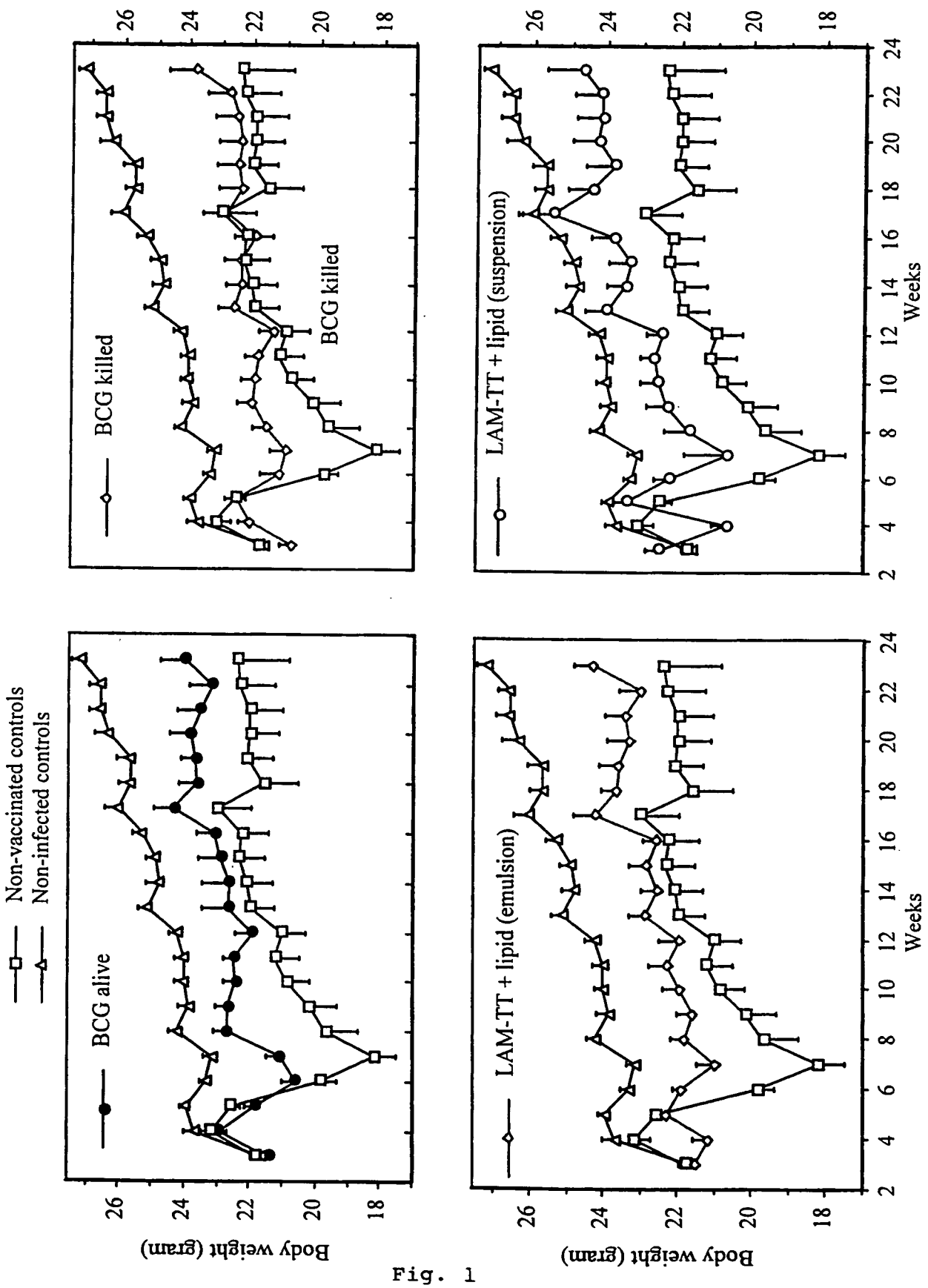

Non-vaccinated controls
Non-infected controls
BCG alive
BCG killed
BCG killed
LAM-TT + lipid (emulsion)
LAM-TT + lipid (suspension)
Body weight (gram)
Body weight (gram)
Weeks
Weeks
18
20
22
24
26
2
4
6
8
10
12
14
16
18
20
22
24

Fig. 1

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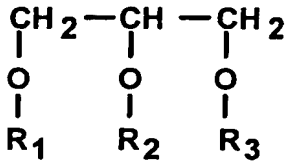
Claims

1. Vaccine formulation against a mycobacterium comprising,

as adjuvant, one or more substances selected from

a) monoglyceride preparations having at least 80 % monoglyceride content and having the general formula



wherein R_1 and R_2 is H and R_3 is one acyl group containing from 6 to 24 carbon atoms, and where the acyl chains may contain one or more unsaturated bonds, in admixture with one or more substances selected from

b) fatty acids of the general formula

$$CH_3 - (CH_2)_n - COOH$$

where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and

as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from *Mycobacterium tuberculosis* which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC).

2. Vaccine formulation according to claim 1, wherein the immunologically active carriers (IAC) contain amino groups and said divalent bridge group has the following structural formula

$$(LAM)\text{-}NH\text{-}\overset{\displaystyle NH_2Cl}{\overset{\|}{C}}\text{-}(CH_2)_3\text{-}S\text{-}CH_2\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-}NH\text{-}(IAC).$$

3. Vaccine formulation according to claim 1 or 2, wherein the adjuvant has a monoglyceride preparation content of at least 90 %, preferably at least 95 %, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more unsaturated bonds, and the immunologically active carriers (IAC) are derived from

polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

4. Vaccine formulation according to any one of claims 1 - 3, which further comprises pharmaceutical excipients selected from the group consisting of biocompatible oils, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

5. Vaccine formulation according to claim 3 or 4, wherein the adjuvant is a mixture of mono-olein and oleic acid, and possibly soybean oil, and the immunizing component is lipoarabinomannan-tetanus toxoid (LAM-TT).

6. Vaccine formulation according to any one of claims 1-5, wherein the formulation is formulated into a preparation for mucosal administration.

7. Vaccine formulation according to claim 6, wherein the mucosal administration is selected from nasal, pulmonary, oral, rectal and vaginal administration.

8. Aerosol or spray package comprising a tuberculosis vaccine composition according to any one of the claims 1 - 7.

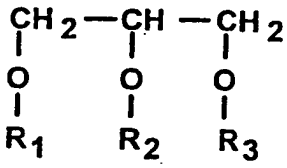
9. Nose-drop package comprising a tuberculosis vaccine composition according to any one of the claims 1 - 6.

10. A method of vaccinating a mammal against a mycobacterium having antigenically active carbohydrate moieties (ACM) derived from *Mycobacterium tuberculosis,* which comprises mucosal administration to the mammal of an protection-inducing amount of a tuberculosis vaccine composition according to any one of claims 1 - 7.

Claims

1. Vaccine formulation against a microorganism comprising,
as adjuvant, one or more substances selected from

a) monoglyceride preparations having at least 80 % monoglyceride content and having the general formula



wherein R_1 and R_2 is H and R_3 is one acyl group containing from 6 to 24 carbon atoms, and where the acyl chains may contain one or more unsaturated bonds and

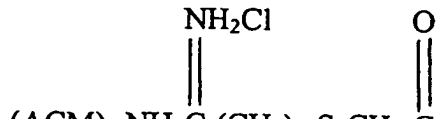
b) fatty acids of the general formula

$$CH_3 - (CH_2)_n - COOH$$

where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and

as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from said microorganism which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC).

2. Vaccine formulation according to claim 1, wherein the immunologically active carriers (IAC) contain amino groups and said divalent bridge group has the following structural formula



3. Vaccine formulation according to claim 1 or 2, wherein the adjuvant has a monoglyceride preparation content of at least 90 %, preferably at least 95 %, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more

unsaturated bonds, and the immunologically active carriers (IAC) are derived from polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

4. Vaccine formulation according to any one of claims 1 - 3, which further comprises pharmaceutical excipients selected from the group consisting of biocompatible oils, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

5. Vaccine formulation according to claim 3 or 4, wherein the adjuvant is a mixture of mono-olein and oleic acid, and possibly soybean oil, and the immunizing component is lipoarabinomannan-tetanus toxoid (LAM-TT).

6. Vaccine formulation according to any one of claims 1-5, wherein the formulation is formulated into a preparation for mucosal administration.

7. Vaccine formulation according to claim 6, wherein the mucosal administration is selected from nasal, pulmonary, oral, rectal and vaginal administration.

8. Aerosol or spray package comprising a TB vaccine composition according to any one of the claims 1 - 7.

9. Nose-drop package comprising a TB vaccine composition according to any one of the claims 1 - 6.

10. A method of vaccinating a mammal against a microorganism having antigenically active carbohydrate moieties (ACM), which comprises mucosal administration to the mammal of an protection-inducing amount of a TB vaccine composition according to any one of claims 1 - 7.